Hypersensitivity to oxygen and shortened lifespan in a *Drosophila* mitochondrial complex II mutant

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Oxidative stress is implicated as a major cause of aging and age-related diseases, such as Parkinson's and Alzheimer's, as well as ischemia-reperfusion injury in stroke. The mitochondrial electron transport chain is the principal source of reactive oxygen species within cells. Despite considerable medical interest, the molecular mechanisms that regulate reactive oxygen species formation within the mitochondrion remain poorly understood. Here, we report the isolation and characterization of a *Drosophila* mutant with a defect in subunit b of succinate dehydrogenase (SDH; mitochondrial complex II). The *sdhB* mutant is hypersensitive to oxygen and displays hallmarks of a progeroid syndrome, including early-onset mortality and age-related behavioral decay. Pathological analysis of the flight muscle, which is amongst the most highly energetic tissues in the animal kingdom, reveals structural abnormalities in the mitochondria. Biochemical analysis shows that, in the mutant, there is a complex II-specific respiratory defect and impaired complex II-mediated electron transport, although the other respiratory complexes remain functionally intact. The complex II defect is associated with an increased level of mitochondrial hydrogen peroxide production, suggesting a possible mechanism for the observed sensitivity to elevated oxygen concentration and the decreased lifespan of the mutant fly.

aging | hyperoxia | reactive oxygen species | *sdhB* | succinate dehydrogenase

The ability to use oxygen for respiration has allowed a vast expansion of the diversity of life. However, as a byproduct of metabolism, reactive oxygen species (ROS) are produced, which are highly toxic to cells, contributing to the etiology of various neurodegenerative disorders (1). Since Harman's initial formulation of the free radical theory of aging (2), much attention has also focused on the possible role of ROS-induced oxidative damage as a primary cause of aging. Age-related accumulation of oxidative damage to cellular macromolecules is observed throughout the animal kingdom (3). Long-lived mutants identified in yeast, worm, fly, and mouse often show enhanced resistance to oxidative stress (4). In both flies and mice, transgenic overexpression of antioxidant enzymes has been reported to increase lifespan (5–7). The same has been demonstrated in flies on decreasing oxidative damage within the nervous system by targeted overexpression of a mitochondrial uncoupling protein (8).

An estimated 90% of cellular ROS are produced by the oxidative phosphorylation system of mitochondria (4), which uses the products of both nuclear and mitochondrial genes to generate cellular ATP. The proteins of the system are organized in five large complexes, electron transport chain complexes I-IV and ATP synthetase (complex V) (9). In the process of mitochondrial electron transport, highly toxic ROS can be produced if single electrons are inappropriately transferred to oxygen, a phenomenon referred to as "electron leakage." Although the major sites of electron leakage have been proposed to be complex I (NADH dehydrogenase) and complex III (cytochrome bc_1) (4), it has been shown, in *Caenorhabditis elegans*, that a mutation in *mev-1*, which encodes subunit C of complex II (succinate dehydrogenase), leads to increased production of ROS (10, 11). Mitochondria are not only sources but also vulnerable targets of ROS (4). It has therefore been proposed that oxidative damage to oxidative phosphorylation components themselves may stimulate further oxidant production, a vicious cycle that can lead to eventual mitochondrial collapse and cell death (3).

With the growing body of evidence supporting causal relationships between mitochondrial dysfunction, ROS, and a wide range of pathological disorders, a better understanding is needed of how individual oxidative phosphorylation components influence respiratory chain activity, ROS formation, and generate a complex array of disease symptoms. In *C. elegans*, RNAi screens to identify genes that influence lifespan identified several components of the oxidative phosphorylation machinery (12, 13). The potential contribution of *Drosophila* genetics, however, has been largely neglected. Fly models of respiratory chain deficiencies could provide a genetically favorable system for dissecting the molecular mechanisms of mitochondria-related disorders. We describe such a study for subunit B of complex II.

Results

Mutation in Succinate Dehydrogenase Subunit b (*sdhB*) Confers Hypersensitivity to Hyperoxia. As the formation of ROS increases with ambient oxygen concentration (14), exposure to hyperoxia (100% O_2) offers an attractive paradigm for physiological studies of oxidative stress (15, 16). We performed a genetic screen for mutations that cause decreased survival under hyperoxia. Among a collection of P-element insertion mutants (17), we identified a hyperoxia-sensitive line, EY12081, which has an insertion in the *sdhB* gene, located at position 42D4 on the second chromosome. Under hyperoxia, the mean survival time of $sdhB^{EY12081}$ flies is reduced to 10% of that of normal flies (Fig. 1*A*). Our molecular analysis of the P-element insertion site from $sdhB^{EY12081}$ flies localized the transposon within the 5′ untranslated region (UTR) of the gene (Fig. 1*B*), consistent with data given by the Gene Disruption Project (http://flypush.imgen.bcm.tmc.edu/pscreen/). An 8-bp sequence, normally present in one copy at the insertion site, is duplicated, flanking both ends of a 10.9-kilobase P-element insertion. Using reverse transcriptase PCR (RT-PCR), we found the *sdhB* transcript to be greatly reduced in $sdhB^{EY12081}$ flies (Fig. 1*C*).

To test whether the observed oxygen sensitivity is caused by the P-element insertion, we generated lines in which the P-element was excised from the insertion site by crossing

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Abbreviation: ROS, reactive oxygen species.

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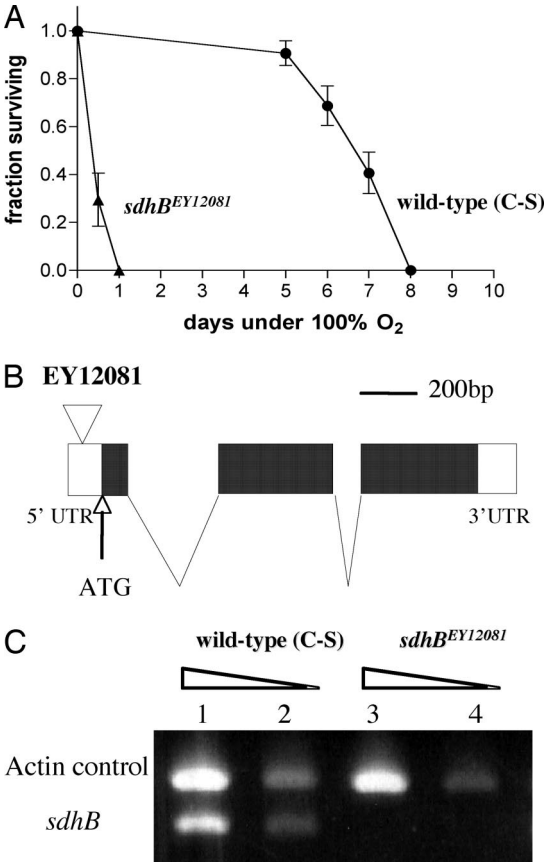

Fig. 1. *Drosophila sdhB* mutant is dramatically sensitive to hyperoxia. (*A*) Survival curves (±SEM) of $sdhB^{EY12081}$ and the wild-type strain Canton-S (C-S) under hyperoxia (100% O_2). $n > 25$ flies; log-rank test; $P < 0.0001$. (*B*) Structure of the *sdhB* gene, with triangle indicating the insertion site of the 10.9-kb P-element (EY12081). Open and hatched blocks indicate untranslated and coding sequences, respectively. (*C*) Transcription of the *sdhB* gene is greatly reduced in the mutant. RT-PCR experiments were performed by using total RNA from wild-type (C-S) (lanes 1 and 2) and *sdhB* mutant (lanes 3 and 4) flies. Shown are RT-PCR products from 10-fold dilutions of sample. Actin served as an internal control.

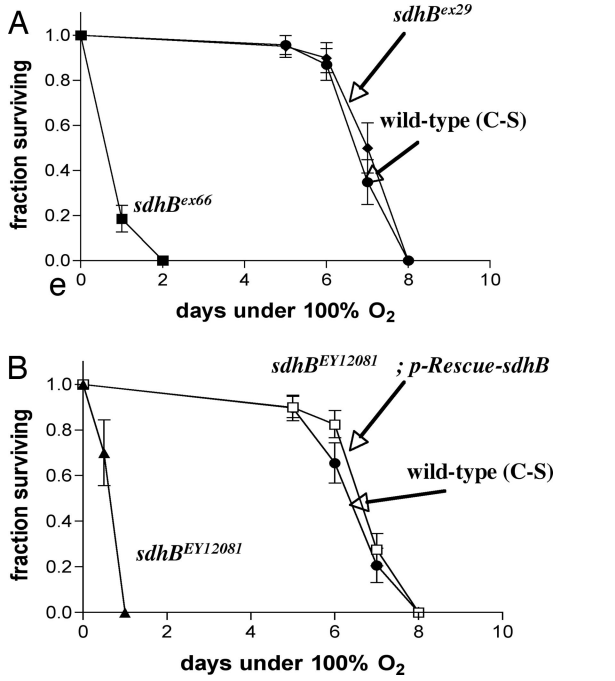

Fig. 2. Genetic rescue of the *sdhB* mutant hypersensitivity to oxygen. (*A*) Precise excision ($sdhB^{ex29}$) of the P-element returns the oxygen resistance to normal. Seven additional, independent, precise excision alleles also fully reverted the mutant phenotype. $sdhB^{ex66}$, a mutant allele, generated by imprecise excision of the P-element, retains the oxygen sensitivity ($n > 25$; log-rank test; $P < 0.0001$). The same was true for four independent, imprecise excision alleles. (*B*) Genomic rescue of the *sdhB* mutant phenotype. Survival curves (±SE) under hyperoxia (100% O_2) of $sdhB^{EY12081}$, the wild-type strain (C-S), and $sdhB^{EY12081}$ flies carrying a transgenic genomic DNA rescue fragment (P-rescue-*sdhB*). This fragment consisted of the *sdhB* transcription unit plus 5 kb of upstream genomic DNA. The genomic rescue construct is sufficient to rescue the $sdhB^{EY12081}$ mutation ($n > 25$; log-rank test; $P < 0.0001$). These data confirm that the mutant phenotype is due to a defect in the *sdhB* gene.

$sdhB^{EY12081}$ to flies harboring a transposase. Eight precise-excision lines fully reverted the oxygen-sensitive phenotype. An example is shown in Fig. 2*A*. This procedure also generated several imprecise excision alleles that retained the oxygen-sensitive phenotype. One of those, $sdhB^{ex66}$, in which 0.4 kilobases of the 3′ P-element sequence remained at the insertion site, was selected for further study. This mutant allele failed to complement the oxygen-sensitivity of $sdhB^{EY12081}$, indicating that the mutations cause defects in the same functional gene. By P-element transformation, transgenic flies (P-rescue-*sdhB*) were generated containing a genomic DNA rescue fragment, consisting of the *sdhB* transcription unit plus 5 kb of upstream genomic DNA. This transgene was, indeed, sufficient to restore normal hyperoxia-resistance to *sdhB* mutant flies (Fig. 2*B*). Both the excision analysis and the rescue data show that the lack of *sdhB* function is responsible for the hypersensitivity to oxygen observed in the mutant.

Mitochondrial Ultrastructural Abnormalities in *sdhB* Mutants Under Hyperoxia. We next examined the consequences of the *sdhB* mutation on flight muscle mitochondrial ultrastructure. The mitochondria typically lie in rows between the myofibrils (Fig. 3*A*). The cristae within these mitochondria are very densely packed so that any changes in their configuration are easily seen, as exemplified by the *Drosophila* mutant *hyperswirl* (15). Electron micrographs of young wild-type flight muscles revealed normal mitochondrial ultrastructure even after 15 h under hyperoxia (Fig. 3*C*). *sdhB* mutants, of the same age, do not display major defects in the flight muscle mitochondria (Fig. 3*B*) but after 15 h of hyperoxia show severe mitochondrial pathology (Fig. 3*D*). The majority of mitochondria are severely damaged and their cristae in disarray, whereas the myofibrils appear unaffected.

Examination of the *sdhB* Mutant Under Normoxia. Under normoxic conditions, mutant flies displayed a 66% decrease in mean survival time and a 17% decrease in maximum survival time (Fig. 4*A*). We next assayed for behavioral correlates of premature aging in the mutant flies. Negative geotaxis, an innate escape response in which flies climb the wall of a container after being tapped down to the bottom, is a commonly assessed *Drosophila* behavior that has been shown to decline with age (18). We tested whether $sdhB^{EY12081}$ mutants displayed an early decline. At 2 days of age, $sdhB^{EY12081}$ mutant flies performed normally, but there was a rapid decay with age. At 8 days, the mutant flies displayed a 54% decrease, and at 12 days he mutant flies displayed a 77% decrease in climbing score (Fig. 4*B*), whereas normal flies showed no decline in that time.

***sdhB* Mutant Displays a Complex II-Specific Respiratory Defect, Impaired Complex II-Mediated Electron Transport, and Increased Mitochondrial ROS Production.** Mitochondrial complex II is the only respiratory chain complex that is completely encoded by nuclear

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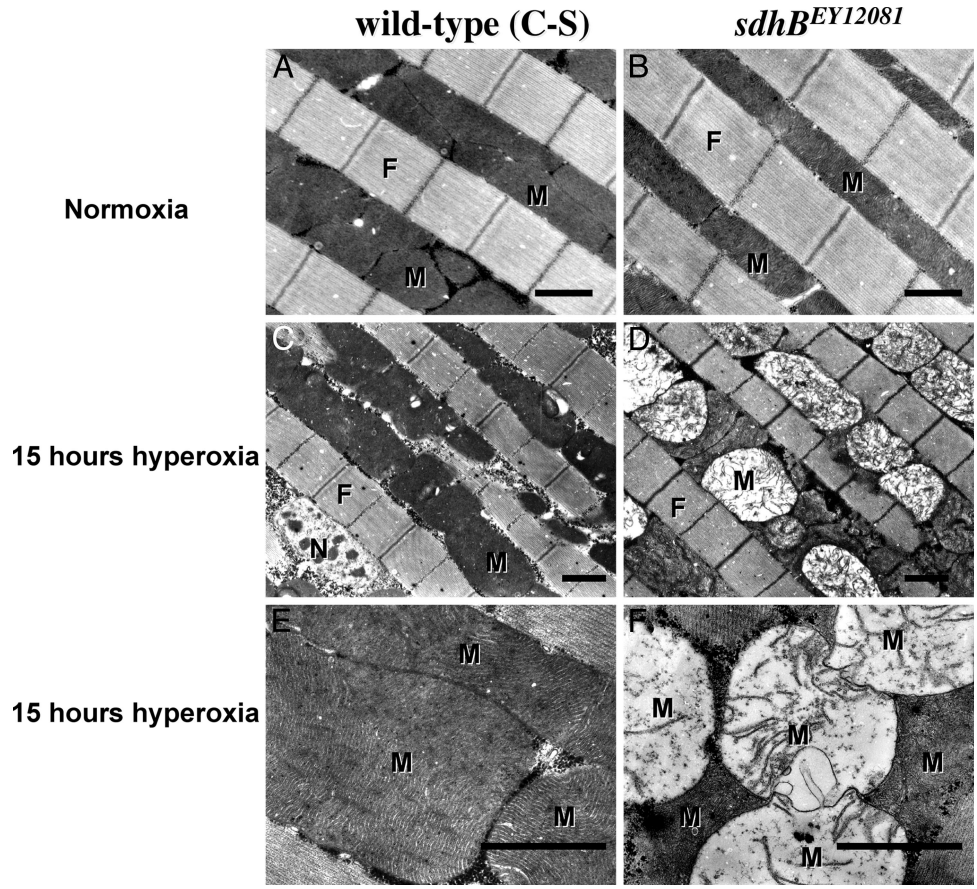

Fig. 3. Hyperoxia causes mitochondrial ultrastructural abnormalities in *sdhB* mutants. Electron micrographs of flight muscle of 7-day-old wild-type (C-S) with (*C*) and without (*A*) 15-h hyperoxia, show normal mitochondrial ultrastructure. Seven-day-old $sdhB^{EY12081}$ under normoxia (*B*) show normal mitochondrial ultrastructure, but, after 15 h under hyperoxia (*D*), the majority of mitochondria are severely damaged, their cristae in disarray. (*E* and *F*) Higher magnification, showing abnormal pattern of cristae in the mutant. (Scale bars, 1 μm.) M, mitochondrion; F, myofibril; *n*, nucleus. The damage in individual mitochondria appears to be a largely all-or-none phenomenon. Note also that the outer membranes of the affected mitochondria are still intact, in contrast to the mitochondrial lesions seen in the mutant *hyperswirl* (15).

genes. It consists of four subunits: SDHA, SDHB, SDHC, and SDHD (19). The genes *sdhA* and *sdhB* encode, respectively, the catalytic flavoprotein and iron-sulfur proteins, whereas *sdhC* and *sdhD* encode hydrophobic, integral membrane proteins that link the catalytic subunits to the matrix side of the mitochondrial inner membrane. Complex II is also a functional member of the citric acid cycle, coupling oxidation of succinate to fumarate in the mitochondrial matrix with reduction of ubiquinone in the membrane. The 30-kDa SDHB subunit contains three iron-sulfur centers that are believed to be important for mediating that function (19).

To analyze the effect of the *sdhB* mutation on respiratory chain activity, the rate of oxygen consumption was measured by using a Clark-type oxygen electrode. The steps in respiration were compared in mitochondria isolated from wild-type and from $sdhB^{EY12081}$ mutant flies by using substrates and inhibitors specific to each complex. The data are shown in Fig. 5*A*. Complex I-specific (pyruvate/malate-dependent/rotenone-sensitive) respiration rate was normal in the *sdhB* mutant, as was the complex IV-specific (ascorbate/TMPD-dependent/KCN-sensitive) respiration rate. However, there was a 56% decrease in the complex II-specific (succinate-dependent/antimycin A-sensitive) respiration rate.

To determine the effect of the *sdhB* mutation on complex II-mediated electron transfer, that activity was assayed on isolated mitochondria by malonate-sensitive succinate–cytochrome *c* reductase activity (20). As compared with wild-type controls, activity in $sdhB^{EY12081}$ mutant mitochondria was reduced by 40% (Fig. 5*B*). The effect of the mutation on ROS production was assayed by mitochondrial hydrogen peroxide production. Mitochondria from $sdhB^{EY12081}$ mutant animals had a 32% increase in hydrogen peroxide production, as compared with wild-type controls (Fig. 5*C*).

Discussion

We have isolated and characterized a *Drosophila* mutant with a defect in the iron-sulfur subunit (*sdhB*) of complex II. Our data indicate that SDHB is critical in preventing electron leakage from complex II, so that mutant animals suffer from increased oxidative stress and, as a result, are highly sensitive to oxygen and die rapidly.

Complex II has not usually been associated with electron leakage and the subsequent generation of ROS under normal physiological conditions, the favored candidates having been complexes I and III (4). A structural basis for low complex II-associated ROS production in normal mitochondria has been attributed to certain amino acid residues within SDHB (21). Specifically, the three iron-sulfur clusters [2Fe-2S], [4Fe-4S], and [3Fe-4S] have been implicated as efficient mediators of electron transfer (19). Our data support the idea that SDHB contains critical sites for electron transfer, because a mutation leading to a decrease in *sdhB* expression leads to electron leakage. In *C. elegans*, a mutation in a different subunit,

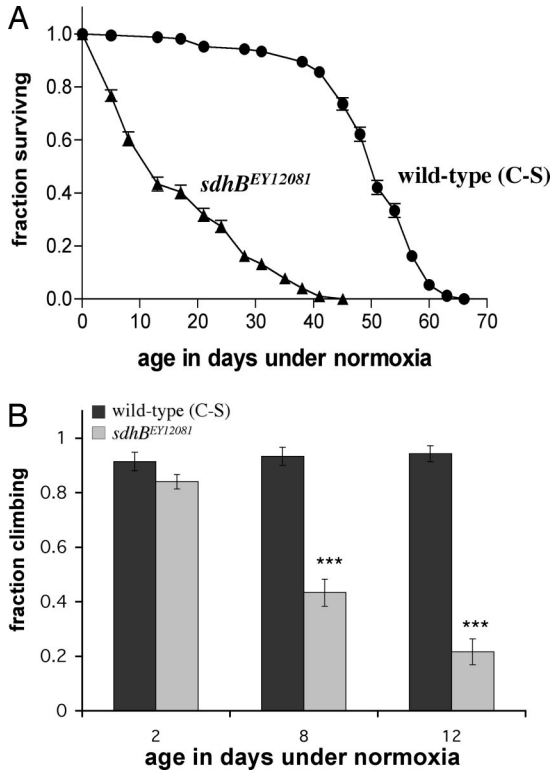

Fig. 4. Under normoxia, the *sdhB* mutant displays reduced lifespan and an age-related decline in climbing ability. (*A*) Survival curves (±SEM) of *sdhB*^{EY12081} mutants and wild-type (C-S). Flies were maintained at 25°C, transferred every 2–4 days, and scored for survival. Even under normoxia, the *sdhB*^{EY12081} mutants were short-lived ($n > 300$ flies; log-rank test, $P < 0.0001$). (*B*) Climbing ability score. At 2 days of age, *sdhB*^{EY12081} mutant flies performed normally ($n > 60$ flies; $P = 0.6$), but, with age, there was a rapid decay to 46% by 8 days ($n > 50$ flies; $P < 0.001$) and 23% by 12 days ($n > 50$ flies; $P < 0.001$).

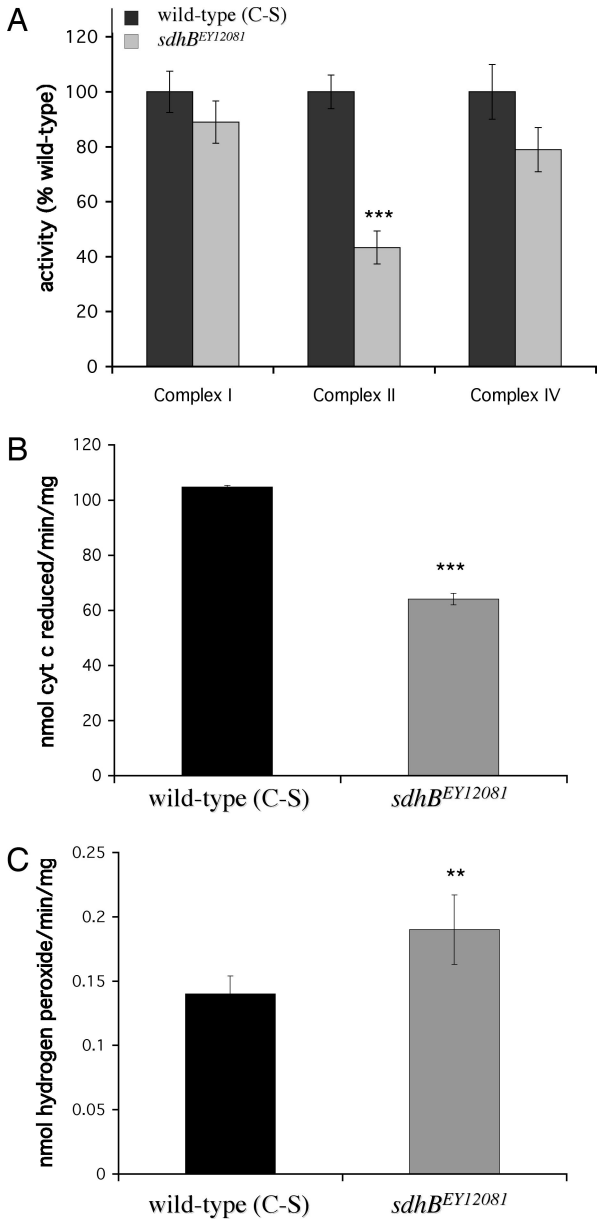

Fig. 5. *sdhB* mutant fly mitochondria display a complex II-specific respiratory defect, reduced complex II electron transport activity and increased H_2O_2 production. (*A*) Oxygen consumption compared in mitochondria isolated from wild-type (C-S) and *sdhB*^{EY12081} mutant flies by using an oxygraph and complex-specific substrates and inhibitors. The mutant showed a 56% decrease in complex II-specific respiration ($n = 5$, $P < 0.0001$). In contrast, complex I-specific respiration, as well as complex IV-specific respiration, showed no significant differences ($n = 5$, $P = 0.32$ and $n = 5$, $P = 0.09$, respectively). Complex III was not measured. (*B*) Complex II-mediated electron transport activity, assayed by malonate-sensitive succinate-cytochrome *c* reductase activity on mitochondria from wild-type (C-S) and mutant flies. Activity was assayed per milligram of protein. Activity in *sdhB*^{EY12081} was reduced by 40% ($n = 3$, $P < 0.0001$). (*C*) Hydrogen peroxide production was measured by using the Amplex Red hydrogen peroxide assay kit (Molecular Probes). Activity was assayed per milligram of protein. Mitochondria from *sdhB*^{EY12081} mutant animals showed a 32% increase in hydrogen peroxide production, as compared with controls from wild-type (C-S) ($n = 12$, $P = 0.02$).

SDHC, leads to ROS overproduction (10). SDHC is a hydrophobic integral membrane protein, which links the catalytic subunits (SDHA and SDHB) to the mitochondrial inner membrane. One possibility is that the SDHC mutation manifests its effect via its interaction with SDHB, because mutations in SDHC or SDHB alone are sufficient, raising an interesting question regarding the molecular interactions between these components.

Mitochondrial respiratory complex II, as a member of the citric acid cycle, oxidizes succinate to fumarate and passes the electrons directly into the quinone pool, serving as the only direct link between activity in the citric acid cycle and electron transport in the membrane. Finer details of these reactions and interactions are poorly understood. Hereditary deficiencies of the mitochondrial electron transport chain are associated with a broad range of human diseases, ranging from myo- and encephalopathies to aging and tumor formation (1, 22). Mutations in individual subunits of complex II result in a range of disorders (23). Whereas defects in SDHA are a cause of Leigh syndrome (24), a severe encephalomyopathy characterized by bilaterally symmetrical necrotic lesions in subcortical brain regions, defects in SDHB, SDHC, and SDHD are the cause of hereditary paragangliomas and pheochromocytomas (25). Paragangliomas are rare, mostly benign, tumors that arise from a component of the neuroendocrine system and are characterized by the development of highly vascular, slow-growing tumors in the head and neck. The carotid body, the largest of all paraganglia, is also the most common site of the tumors. The pheochromocytomas are catecholamine-producing, chromaffin tumors that in 90% of cases arise in the adrenal medulla. Although an increase in mitochondrial ROS

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production has been suggested as a possible underlying cause (19), understanding of these diseases has been limited by a dearth of animal models carrying mutations in each of the complex II subunits.

This mutant is an animal model of both the biochemical and phenotypic consequences of SDHB deficiency. The *Drosophila sdhB* model also provides a system with which to screen for suppressor genes and drugs that might ameliorate the damaging effects of elevated mitochondrial ROS.

Materials and Methods

Fly Strains. Male flies were used throughout the experiments. The wild-type strain was *Canton-S* (C-S). We screened a publicly available collection of P-element insertion lines (17) for mutants sensitive to hyperoxia and identified such an insertion in *sdhB*. To create *sdhB* excision alleles, single *w; sdhB*^{EY12081}*/CyO w+Hop2.1* males were crossed to *w;Sco/CyO*, and *w-* male progeny were used to establish stocks.

Lifespan Under Hyperoxia. Adult males (3- to 4-days-old) in cotton-stoppered, 23 mm × 95 mm, shell vials (20–25 flies per vial) on lab-standard food (cornmeal/sucrose/agar/yeast) (26) were placed in a Plexiglas enclosure of 28 × 28 × 24 inches at room temperature (22–24°C). Oxygen (100%) was passed through the box at a constant rate (300 ml/min). Each experiment was conducted on at least 50 flies of a given genotype. Some flies were removed after 15 h for EM studies.

Lifespan Under Normoxia. Newly eclosed flies were maintained at 25°C in cotton-stoppered shell vials (20–25 flies per vial) on lab standard food, transferred to fresh vials every 2–3 days, and scored for survival. Each experiment was conducted with at least 200 flies of each genotype.

Electron Microscopy. Dorsal indirect flight muscle was dissected from decapitated adult flies at 4°C in 2% paraformaldehyde plus 1% glutaraldehyde, fixed overnight, then postfixed in 1% osmium tetroxide at room temperature, dehydrated in an ethanol series, and embedded in Epon 812. Ultrathin sections (80 nm) were examined with a 420 electron microscope (Philips, Eindhoven, The Netherlands) at 100 kV.

RT-PCR. Total RNA was extracted from 40 flies by using TRIzol reagent (Invitrogen, Carlsbad, CA), concentration was measured by using a Nanodrop spectrophotometer, and sample concentrations were normalized. The Retroscript kit (Ambion, Austin, TX) was used according to the manufacturer's instructions, with the oligo(dT) primers provided. The *sdhB* cDNA was amplified by PCR by using primers (F, 5′-CCACACTGCACCCTCAGTTT-3′; R, 5′-GCGGTACTGCTCGTAGAAGTTG-3′) specific for a single 600-bp amplicon. Actin cDNA was amplified to confirm normalization.

Genomic Rescue. Seven kilobases of genomic DNA was amplified by high-fidelity PCR from wild-type flies by using the forward primer 5′-CCTCCTTTAAAGCTACCTGTGC-3′ and the reverse primer 5′-GCGTCTTGTTCCCTTTACAAAC-3′. The resulting PCR product was cloned into TOPO-pCR-XL3.1 (Invitrogen) to give pTOPO-*sdhB*. The coding region of *sdhB* was then sequenced in pTOPO-*sdhB* to verify that no mutations had been introduced. The pTOPO-*sdhB* vector was linearized with NotI and ligated into the NotI digested P element transformation vector pCASPER to give pRescue-*sdhB*. The purified pRescue-*sdhB* vector was subsequently injected into w^{1118} embryos, and a third chromosome insertion line was selected for further study.

Climbing Performance Assay. Approximately 15 flies were placed in a cotton-stoppered plastic vial (23 mm × 95 mm), gently tapped to the food at the bottom, and the number of flies that reached the top third of the vial after 20 s was measured. Each experiment was conducted on at least 40 flies of each genotype, and the data were averaged.

Isolation of Mitochondria. Mitochondria were isolated from adult flies as described by Schwarze *et al.* (27). Fifty flies were gently crushed in 1 ml chilled mitochondrial isolation medium (MIM: 250 mM sucrose, 10 mM Tris (pH 7.4), 0.15 mM $MgCl_2$) by using a glass-on-glass homogenizer, then spun twice at 1,000 × *g* for 5 min at 4°C to remove debris. The supernatant was then spun at 13,000 × *g*, for 5 min at 4°C. The pellet, containing the mitochondria, was washed with 1 ml MIM and resuspended in 50 μl MIM.

Complex II Activity. The ability of complex II to catalyze electron transport was assayed at 37°C by malonate-sensitive succinate-cytochrome *c* reductase activity (20) in 1.0 ml 0.1 M Tris (pH 7.4), containing 1.6 mg cytochrome *c*, 1 mM sodium cyanide, and 20 mM sodium succinate. The reference cuvette contained the same solution, plus 20 μl of 20% sodium malonate (a specific inhibitor of complex II) solution.

Complex-Specific Respiration. Rate of oxygen consumption was measured polarographically by using a Clark-type oxygen electrode connected to a computer-operated Oxygraph control unit (Hansatech Instruments, Norfolk, England) at 27°C. The sample was freshly isolated mitochondria, prepared, as described above, from 50 flies, suspended in 120 mM KCl, 5 mM K_2HPO_4, 3 mM Hepes, 1 mM EGTA, 1 mM $MgCl_2$, and 0.2% BSA (pH 7.2). The rate was measured continuously, with the following sequential additions: 5 mM pyruvate plus 5 mM malate, 50 μM dinitrophenol (DNP), 400 nM rotenone, 10 mM succinate, 200 nM antimycin A, 10 mM ascorbate plus 400 μM N′-tetramethyl-1,4-phenylenediamine (TMPD; Fluka, St. Gallen, Switzerland), 2 mM KCN. The concentration of DNP specified above was chosen by preliminary tests for maximal stimulation of respiration rate in the presence of pyruvate and malate (data not shown). The concentrations of rotenone and antimycin A were the minimal concentrations that achieved maximal inhibition of respiration rate in the presence, respectively, of pyruvate plus malate, or succinate. DNP-stimulation of respiration rate in the presence of pyruvate plus malate was used to assess the degree of coupling of the respiration in the mitochondrial preparation. Pyruvate/malate-dependent respiration was calculated as the rotenone-sensitive rate of oxygen consumption in the presence of DNP, pyruvate, and malate. The succinate-dependent respiration was calculated as the antimycin A-sensitive rate of oxygen consumption in the presence of DNP, pyruvate, malate, rotenone, and succinate. The ascorbate/TMPD-dependent respiration rate was calculated as the KCN-sensitive rate of oxygen consumption in the presence of DNP, pyruvate, malate, rotenone, succinate, antimycin A, ascorbate, and TMPD. The substrate-dependent respiration rates were measured in the presence of DNP. Therefore, they represent maximal uncoupled respiration rates.

Mitochondrial Hydrogen Peroxide Production. Mitochondria were isolated from 7-day-old flies as described above. Production of hydrogen peroxide was measured as described by Fridell *et al.* (8) by using the Amplex Red hydrogen peroxide assay kit (Molecular Probes, Eugene, OR).

Statistical Analysis. Survival curves were analyzed by using the Prism4 software (Graphpad), and the results were reported as mean lifespan ± SEM. The *P* values given are the results of a logrank test

on the Kaplan-Meier data. For the bar charts, error bars represent SEM, and the *P* values are based on the Student's *t* test.

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1. Wallace DC (2005) *Annu Rev Genet* 39:359–407.
2. Harman D (1957) *J Gerontol* 2:298–300.
3. Shigenaga MK, Hagen TM, Ames BN (1994) *Proc Natl Acad Sci USA* 91:10771–10778.
4. Balaban RS, Nemoto S, Finkel T (2005) *Cell* 120:483–495.
5. Parkes TL, Elia AJ, Dickinson D, Hilliker AJ, Phillips JP, Boulianne GL (1998) *Nat Genet* 19:171–174.
6. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, *et al.* (2005) *Science* 308:1909–1911.
7. Sun J, Folk D, Bradley TJ, Tower J (2002) *Genetics* 161:661–672.
8. Fridell YW, Sanchez-Blanco A, Silvia BA, Helfand SL (2005) *Cell Metab* 1:145–152.
9. Saraste M (1999) *Science* 283:1488–1493.
10. Senoo-Matsuda N, Yasuda K, Tsuda M, Ohkubo T, Yoshimura S, Nakazawa H, Hartman PS, Ishii N (2001) *J Biol Chem* 276:41553–41558.
11. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, Suzuki K (1998) *Nature* 394:694–697.
12. Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) *Science* 298:2398–2401.
13. Lee SS, Lee RY, Fraser AG, Kamath RS, Ahringer J, Ruvkun G (2003) *Nat Genet* 33:40–48.
14. Turrens JF (2003) *J Physiol* 552:335–344.
15. Walker DW, Benzer S (2004) *Proc Natl Acad Sci USA* 101:10290–10295.
16. Walker DW, Muffat J, Rundel C, Benzer S (2006) *Curr Biol* 16:674–679.
17. Bellen HJ, Levis RW, Liao G, He Y, Carlson JW, Tsang G, Evans-Holm M, Hiesinger PR, Schulze KL, Rubin GM, *et al.* (2004) *Genetics* 167:761–781.
18. Grotewiel MS, Martin I, Bhandari P, Cook-Wiens E (2005) *Ageing Res Rev* 4:372–397.
19. Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D, Bartlam M, Rao Z (2005) *Cell* 121:1043–1057.
20. Robinson KM, Lemire BD (1995) *Methods Enzymol* 260:34–51.
21. Yankovskaya V, Horsefield R, Tornroth S, Luna-Chavez C, Miyoshi H, Leger C, Byrne B, Cecchini G, Iwata S (2003) *Science* 299:700–704.
22. Larsson NG, Rustin P (2001) *Trends Mol Med* 7:578–581.
23. Ackrell BA (2002) *Mol Aspects Med* 23:369–384.
24. Bourgeron T, Rustin P, Chretien D, Birch-Machin M, Bourgeois M, Viegas-Pequignot E, Munnich A, Rotig A (1995) *Nat Genet* 11:144–149.
25. Bayley JP, Devilee P, Taschner PE (2005) *BMC Med Genet* 6:39.
26. Lewis EB (1960) *Drosophila Information Service* 34:118–119.
27. Schwarze SR, Weindruch R, Aiken JM (1998) *Free Radic Biol Med* 25:740–747.